# ELECTROPHORETIC VARIATION OF PHOSPHOGLUCOMUTASE, GLUCOSE-6-PHOSPHATE, HEXOSE-6-PHOSPHATE AND TETRAZOLIUM OXIDASE ENZYMES IN SOME FISHES FROM KHOR AL-ZUBAIR, IRAQ AND KUWAIT

by

L.A.J. AL-HASSAN, A.A. MAHDI & L.I. IBRAHIM (1)

ABSTRACT.– Tissue extracts of skeletal muscle and liver were electrophoretically examined for phosphoglucomutase, (PGM ; EC. 2.7.5.1), glucose-6-phosphate dehydrogenase (G6PD), hexose-6-phosphate dehydrogenase (H6PD) and tetrazolium oxidase (TO) activity in thirty two species of teleostean fishes. One phosphoglucomutase, glucose-6-phosphate dehydrogenase, hexose-6-phosphate dehydrogenase and tetra zolium oxidase were found in all the teleost groups studied. In the fishes studied tetrazolium oxidase appeared to be controlled by two co-dominant alleles, except in *Therapon puta* in which this enzyme appeared to be controlled by three alleles. TO, G6PD, and H6PD can be considered as good taxonomic criteria to differentiate *Johnius aneus* from the remaining fish species. On the other hand PGM can be considered as a good taxonomic criterion to differentiate *Lutjanus fulviflamma*.

RÉSUMÉ. – Des extraits de muscle et de foie ont été examinés par électrophorèse pour évaluer l'activité en phosphoglucomutase (PGM), glucose-6-phosphate deshydrogenase (G6PD), hexose-6-phosphate deshydrogenase (H6PD) et tetrazolium oxydase (TO), chez trente deux espèces de poissons téléostéens. Une enzyme de chacune de ces catégories a été mise en évidence chez toutes les espèces étudiées. Chez les poissons étudiés, la tetrazolium oxydase semble contrôlée par deux allèles principaux, sauf chez *Therapon puta* où cette enzyme est contrôlée par trois allèles. TO, G6PD et H-6-PD sont de bons critères taxinomiques pour distinguer *Johnius aneus* des autres espèces. Par ailleurs, PGM est un bon critère taxinomique pour distinguer *Lutjanus fulviflamma*.

Mots-clés : Teleostean, ISW, Iraq, ISW Kuwait, Enzyme activity, Electrophoresis, Chemotaxonomy.

Structural variation of homologous proteins has been widely investigated by the technique of electrophoresis. Specific staining methods for enzyme proteins which stimulated research on isozymes (Markert and Moeller, 1959) may now be conveniently used to specifically identify a large number of enzyme proteins on electrophorograms (Shaw and Prasad, 1970).

Phosphoglucomutase (PGM; EC, 2.7.5.1) plays an important role in carbohydrate metabolism. It catalyses a reaction converting glucose-1-phosphate to glucose-6-phosphate. Genetic variation of PGM was first reported in man by Spencer *et al.*

(1) Marine Science Centre, University of Basrah, Basrah-Iraq.

(1964). Genetic variations of PGM also have been described in several animal groups (Martin and Cailovet, 1976; Janson, 1985; Johnson, 1985). Instances of PGM polymorphism have been observed in fishes by Stein *et al.* (1985) and Grant (1984). No previous record for this enzyme has been given for fishes in the Arabian Gulf region, except for that of Al-Hassan (1985) in which PGM was studied from several freshwater fishes from Iraq.

Tetrazolium oxidase is an emperical designation for an enzyme first described by Brewer (1967). It is demonstrated in most animal tissues when tetrazolium dyes are used to detect the electrophoretic isozyme pattern of a variety of dehydrogenases. It catalyses the transfer of electrons from reduced tetrazolium dyes to oxygen in the absence of added cofactors and is detected as chromatic areas against the coloured background of the reduced dye.

The genetic variants of this enzyme have been studied in fishes (Numachi, 1972 ; Iwata, 1973). To our knowledge there is no previous work on this enzyme in the fishes of the Arabian Gulf area. Glucose-6-phosphate dehydrogenase (EC. 1.1.1.49) has been the subject of two recent reports (Cederbaum and Yoshida, 1976 ; Cruz *et al.*, 1982). Most work on this enzyme is based on the Salmonidae, especially rainbow trout, *Salmo gairdneri* (Ohno *et al.*, 1966 and Kamada and Hori, 1970), and brook trout, *Salvelinus fontinalis.*

Hexose-6-phosphate catalyses the oxidation of glucose-6-phosphate, galactose-6-phosphate, and 2-dehydroxy glucose-6-phosphate. It can be distinguished from glucose-6-phosphate by many methods, one of them being electrophoresis (Shaw and Koen, 1968). It is found in several tissues, but its activity is generally greatest in the liver (Mandula *et al.*, 1970). This enzyme has been studied in various species of fishes (Stegeman and Goldberg, 1971 ; Stegeman, 1972).

In this paper evidence is presented for the electrophoretic variability of phosphoglucomutase, tetrazolium oxidase, glucose-6-dehydrogenase and hexose-6-phosphate dehydrogenase in some marine fishes from the Arabian Gulf. We also discuss the possibility of using these enzymes as taxonomic criteria to differentiate the species of fishes under considération.

## MATERIALS AND METHODS

Samples of fishes used in this study were obtained from the commercial catch at Kuwait fish market and from the monthly sampling programme of the Marine Science Centre, University of Basrah (Iraq) at Khor Al-Zubair, north west of the Arabian Gulf. The species of fishes were arranged systematically (following Romer, 1966) and listed in Table I. Extracts for electrophoresis were prepared from separate muscle and liver tissues from the fishes immediately post mortem, and homogenized in a tissue grinder for one minute with an equivalent volume of tris-EDTA borate buffer (pH 8.6). The homogenates were centrifuged for 30 mn at 5000 rpm.

Horizontal starch-gel electrophoresis was carried out using the Tris-borate buffer under previously described conditions (i.e. Miller and El-Tawil, 1974). The

Table I. – Species of fish examined for PGM, TO, G6PDH, and H6PD enzymes

| Super order<br>Order | Family | Scientific name | Sample size |
|---|---|---|---|
| Clupeomorpha<br>Clupeiformes | Clupeidae | *Ilisha elongata*<br>*Nematolosa nasus* | 20<br>20 |
| Acanthopterygii<br>*Scorpaeniformes* | Platycephalidae | *Platycephalus indicus* | 4 |
| Atheriniformes | Belonidae | *Tylosurus strongylurus* | 4 |
| Perciformes | Lutjanidae | *Lutjanus fulviflamma*<br>*L. johni*<br>*L. coccineus* | 4<br>5<br>5 |
| | Lethrinidae | *Lethrinus kallopterus* | 5 |
| | Sciaenidae | *Johnius aneus*<br>*J. caruta*<br>*Otolithus ruber* | 10<br>10<br>10 |
| | Mugillidae | *Liza macrolepis* | 10 |
| | Stromateidae | *Pampus argenteus* | 2 |
| | Theraponidae | *Therapon theraps*<br>*Th. puta* | 4<br>4 |
| | Mullidae | *Upeneus tragula*<br>*Mulloidichthys auriflamma* | 2<br>2 |
| | Siganidae | *Siganus oramin*<br>*S. javus* | 4<br>2 |
| | Carangidae | *Chorinemus lysan*<br>*Caranx kalla* | 2<br>2 |
| | Leiognathidae | *Leiognathus fasciatus* | 5 |
| | Nemipteridae<br>(Subfam. Scolopsinae) | *Scolopsis phaeops* | 5 |
| | Gerridae | *Gerres flamentosus* | 5 |
| | Pomadasyidae<br>(Subfam. Plectorhynchinae) | *Plectorhynchus schotaf*<br>*Plectorhynchus pictus* | 2<br>2 |
| | Sparidae | *Argyrops filamentosus*<br>*Acanthopagrus bifasciatus*<br>*A. luteus* | 4<br>10<br>10 |
| | Scatophagidae | *Scatophagus argus* | 10 |
| Pleuronectiformes | Soleidae | *Synaptura orientalis* | 5 |
| | Cynoglossidae | *Cynoglossus arel* | 5 |

gel contained 13 % starch.

The enzymes PGM, TO and G6PDH were located on the gel by the filter paper method described by Scopes (1968) : the isozymes are transfered to filter paper applied to the surface of the gel, instead of separating on the gel itself. The PGM stain contained 8 mM glucose-1-phosphate, 8 mM glucose-1,6-phosphate, 3 mg/ml nitroblue tetrazolium, 0,5 mg/ml phenazine methosulphate, 0.2 mM NADP and 10 $\mu$ glucose-6-dehydrogenase enzyme. The TO and G6PD enzymes were visualized by using the same stain mixture but differ in containing 8 mM glucose-6-phosphate

and not glucose-6-phosphate dehydrogenase enzyme. The staining mixture was buffered at pH 7.8 with 50 mM Tris and 5 mM magnesium sulphate. The gel was placed in the dark at 37° until the stain had developed.

RESULTS :

*Phosphoglucose mutase :*

Enzymograms of the muscle extract from the fishes under consideration show PGM phenotypes consisting of only one, or both of the two bands A & B, (Fig. 1). The PGM phenotypes may be explained by a two-allele hypothesis, the single-banded phenotypes presumably reflect individuals homozygous for allelic genes and the two-banded phenotype reflect heterozygous fish. The two-banded heterozygous phenotypes suggest that the active enzyme is a monomer.

The skeletal muscle electrophorograms (Fig. 1a) of the species representing each different order show either one or two bands. A polymorphism at PGM locus was observed in *Ilisha elongata, Plectorhynchus schotaf,Plectorhynchus pictus, Caranx kalla, Chorinemus lysan* and *Leiognathus fasciatus.* The isozymes contained in PGM bands of all the species studied show an anodic migration. The slowest anodic was observed in *Caranx kalla, Siganus javus, Lethrinus kallopterus, Leiognathus fasciatus* and *Nematolosa nasus* and the fatest in *Lutjanus fulviflamma.* There was little variation in the mobility of the fast and slow bands, except for the fast band of *Lutjanus fulviflamma* which distinguishes this species from the remaining members of the family Lutjanidae as well as the other species studied.

*Tetrazolium oxidase :*

Bands formed in positive staining tests were located identically to the achromatic zones on starch-gel, indicating similar enzymatic activity to that described by Brewer (1967) and, there-by, verifying that the achromatic zones reflected tetrazolium oxidase activity against a non-specific dark blue background.

The muscle extract from all fish species studied revealed three types of TO designated A, AB, and B (Fig. 1b), *Therapon puta* showed a unique pattern, designated AC. These results suggests that the variation of TO in *Therapon puta* is controlled by three co-dominant alleles, the homozygotes for $TO^B$ allele produce one kind of dimer, BB, migrating faster than the dimeric isozyme of A subunits. In the case of the homozygote for $TO^A$ allele, each of the heterozygotes ($TO^B$ / $TO^A$ and $TO^a$ / $TO^c$) produces two homodimers and one heterodimer.

From the skeletal muscle electrophorogram (Fig. 1b) of the species belonging to the different orders, it is obvious that some species of fishes exhibit a polymorphism at the TO locus ; these are, *Mugil macrolepis, Acanthopagrus latus, Caranx kalla, Therapon puta, Lutjanus coccineus.* The isozymes contained in TO bands of all the species studied shows an anodic migration except for *T. puta.* The fastest

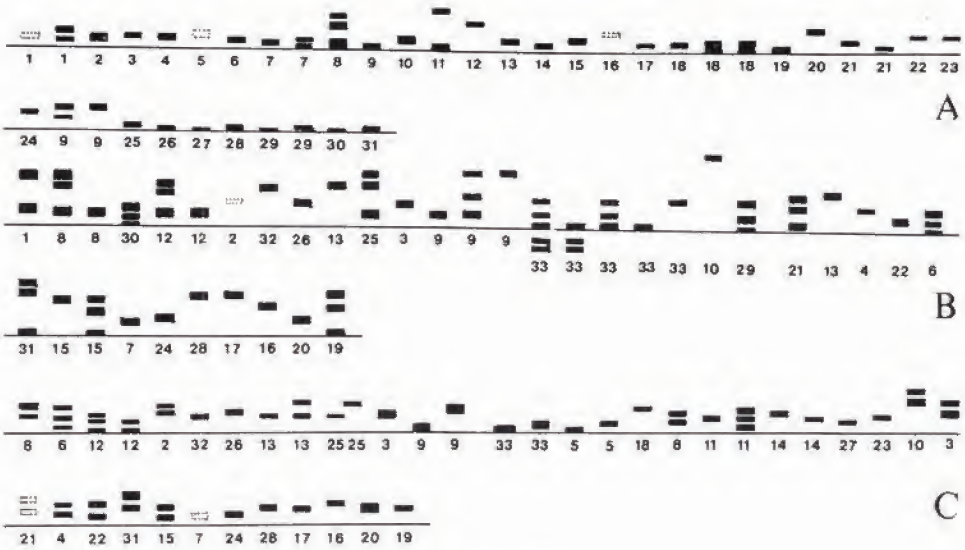


Figure 1. – Diagrams of (a) PGM, (b) TO, (c) G6PDH in :

1. *Ilisha elongata*
2. *Platycephalus indicus*
3. *Tylosurus strongylurus*
4. *Argyrops filamentosus*
5. *Pampus argenteus*
6. *Scatophagus argus*
7. *Plectorhynchus schotaf*
8. *Liza macrolepis*
9. *Caranx kalla*
10. *Johnius aneus*
11. *Lutjanus johni*
12. *Acanthopagrus luteus*
13. *Johnius caruta*
14. *Siganus oramin*
15. *Lutjanus coccineus*
16. *Therapon theraps*
17. *Upeneus tragula*
18. *Plectorhynchus pictus*
19. *Mulloidichthys auriflamma*
20. *Lutjanus fulviflamma*
21. *Chorinemus lysan*
22. *Scolopsis phaeops*
23. *Acanthopagrus bifasciatus*
24. *Otolithus ruber*
25. *Cynoglossus arel*
26. *Siganus javus*
27. *Lethrinus kallopterus*
28. *Synaptura orientalis*
29. *Leiognathus fasciatus*
30. *Nematolosa nasus*
31. *Gerres filamentosus*
32. *Thryssa hamiltonii*
33. *Therapon puta*

anodic band was observed in *Johnius aneus* which makes this species distinguishable from the remaining fish species studied.

*Glucose - 6 - phosphate dehydrogenase :*

The liver electrophorogram of the fish species under consideration is shown in figure 1 (c). The electrophoretic pattern of some species can be divided into three

zones of enzymic activity (*Scatophagus argus, Lutjanus fulviflamma* and *Acanthopagrus latus*). The least anodally migrating zone corresponded to H6Pd, where-as, the remaining two bands were G6P specific. Some other fish species show one or two bands of activity (Fig. 1c). The observed banding patterns suggest two Gd loci, the more mobile band, Gd-1, the less mobile band, Gd-2. The G6PD phenotypes may be explained by a two allele hypothesis-the two single-banded phenotypes presumably reflect individuals homozygous for allelic genes, and the two banded phenotypes reflect heterozygous fish. The enzyme G6PDH is a dimer, although the presence of two-banded heterozygous phenotypes suggests the active enzyme is a monomer (see discussion).

The H6PD phenotypes on the electrophorogram of some species of fish indicate a monomeric structure for this enzyme (*Lutjanus fulviflamma* fig. 1c). No enzymatic activity has been detected on the electrophorogram of *Ilisha elongata, Nematolosa nasus, Leiognathus fasciatus* and *Johnius caruta.*

The isozymes contained in G6PD and H6PD bands of all species studied show an anodic migration. The fastest was observed in *Johnius aneus.* There was no much variation in the mobility of the fast and slow bands. A lower staining intensity was observed in *Chorinemus lysan* which makes it possible for this species to be differentiated from the remaining fish species studied.

**DISCUSSION :**

It is often possible to deduce the genetic control of an enzyme system by studying differences in electrophoretic patterns, even when laboratory breeding is impossible or impractical. All the PGM patterns observed in this study, which included thirty one species of teleostean fishes, can be explained on the basis of the presence of one locus.

Although duplication in the PGM locus was reported in some species of fishes where two or more distinct zones of activity were present on their zymograms (Utter, 1971 and Watts & Watts, 1968), it is apparent from our results that there is no duplication in the PGM locus in fish species belonging to the orders Clupeiformes, Scorpaeniformes, Atheriniformes, Perciformes and Pleuronectiformes. These results complement the findings of Al-Hassan (1985) based on fresh water fishes.

Although two distinct zones of activity for TO were present on the zymogram of some species of fishes (Utter, 1971; Edmunds & Samsons, 1971), it appeared from our results that there is only one zone of activity of TO in the different species of fishes we studied. An hypothesis involving one locus and two alleles can be deduced from the present results except for *Therapon puta* for which the zymogram shows a number of phenotypes that indicate the presence of three alleles. The same results were obtained for an other fish species, *Sebastes inermis* (Numachi, 1972); three alleles were also found in the smooth Washington Clam (Johnson & Utter, 1973).

G6PD isozymes were found in mammals in two forms encoded in two distinct gene loci. One is controlled by a sex-linked locus and is found in all tissues studied, including erythrocytes (Shaw and Koen, 1968). In contrast, the other isozymic form is the product of an autosomal gene locus and occurs in most tissues, but not in erythrocytes (Shaw and Barto, 1965). The possibility that under the conditions of electrophoresis (Tris-borate buffer, pH 8.6) the molecule is in fact in an active dimeric form or, indeed, a monomeric one in disassociation equilibrium at the pH of electrophoresis (Cederbaum and Yoshida, 1976) must be considered. In which case a monomeric-type of behaviour for this enzyme would be detected in the fishes under consideration.

G6PD and H6PD can both oxidize G6P in the presence of NADP as coenzyme. Yet the significant differences in many of their physiochemical properties have led some investigators to consider them to be two different enzymes, rather than isozymes (Mandula *et al.*, 1970).

Stegman and Goldberg (1971) and Srivastava *et al.*, (1972) suggest that G6PD and H6PD arose from a common ancestral type G6PD. Although H6PD has been identified in all classes of vertebrates, it does not seem to be present among the invertebrate species (Powers *et al.*, 1968; Jones, 1970; Kamada and Hori, 1970). Therefore, the progenitor of H6PD probably arose near the beginning of vertebrate evolution, by duplication of the ancestral G6PD gene locus. The absence of this enzymes activity in fishes belonging to the super-order Clupeomorpha may support this hypothesis.

From the skeletal electrophorogram of PGM it is clear that *Lutjanus fulviflamma* can be distinguished from the other fish studied by its fast band mobility.

The electrophorogram of tetrazolium oxidase in *Johnius aneus* and *Therapon puta* shows a distinctive mobility. They can be distinguished from the remaining fish species studied by the anodic mobility of the former and the cathodic of the latter.

G6PD and H6PD on the other hand, succeeded to differentiate *Johnius aneus* from the remaining fish species studied by its anodic mobility.

Together, TO, G6PD and H6PD can be considered as a good taxonomic criterium to differentiate *Johnius aneus* from the other fish studied as part of this investigation. On the other hand, PGM can be considered as a good taxonomic criterion to differentiate *Lutjanus fulviflamma* from other species.

The closeness in the electrophoretic mobility of PGM zones of activity for members of the families Sciaenidae, (*Johnius caruta, J. aneus*), Mullidae (*Upeneus tragula, Mulloidichthys auriflamma*), Siganidae (*Siganus javus, S. oramin*) and Pomadasyidae (*Plectorhynchus schotaf, Plectorhynchus pictus*) might indicate a close relationship between these species. On the other hand, the closeness in the electrophoretic mobility of G6PD zones of activity for members of the families Mullidae (*Upeneus tragula, Mulloidichthys auriflamma*) and Siganidae (*Siganus javus, S. oramin*) might also be indicative of similarities between these species.

REFERENCES

AL-HASSAN, L.A.J., 1985. – Phosphoglucose isomerase and phosphoglucose mutase isozymes in some freshwater fishes from Basrah, Iraq. *Biochem. Syst. Ecol.* 13(3) : 357-364.

BREWER, G.J., 1967. – A-chromatic regions of tetrazolium stained starch Gels : Inherited electrophoretic variation. *Amer. J. Human Genetics*, 19 (5) : 674-680.

CEDERBAUM, S.D. & YOSHIDA, A., 1976. – Glucose-6-phosphate dehydrogenase in rainbow trout. *Biochem. Genetics* 14 (314) : 245-258.

CRUZE, T.A., J.P., THORPE & PULLIN, R.S.V., 1982. – Enzyme electrophoresis in *Tilapia zillii* : A pattern for determining biochemical genetics markers for use in *Tilapia* stock identification. *Aquaculture* 29 : 311-329.

EDMUNDS, P.H. & SAMSONS, J.I., 1971. – Genetic polymorphism of tetrazolium oxidase in Bluefin Tuna, *Thunnus thynnus*, from the Western North Atlantic. *J. Fish. Res. Bd. Canada* 28 : 1053-1055.

GRANT, W.S., 1984. – Biochemical population genetics of Atlantic herring, *Clupea harengus*. *Copeia* 2 : 357-364.

IWATA, M., 1973. – Genetic polymorphism of tetrazolium oxidase in Walleye Pollock. *Jap. J. Genetics* 48 (2) : 147-149.

JANSON, K., 1985. – A morphological and genetic analysis of *Littorina saxatilis* (Prosobranchia) from Venice, and on the problem of *saxatilis rudis* nomenclature. *Biol. J. Linn. Soc.* 24 : 51-59.

JOHNSON, A.G. & F.M. UTTER, 1973. – Electrophoretic variation of adductor muscle protein and tetrazolium oxidase in the smooth Washington Clam, *Saxidomus giganteus* (Deshayes, 1839). *Anim. Blood Grps Biochem. Genet.* 4 : 147-152.

JOHNSON, C., 1985. – Biochemical genetic variation in populations of *Larus argentatus* and *Larus fuscus* in north western Europe. *Biol. J. Linn. Soc.* 24 : 349-363.

JONES, M.L., 1970. – The presence of certain dehydrogenases among polychaetous annelids as shown by disc electrophoresis. *Comp. Biochem. Physiol.* 36 : 605.

KAMADA, T. & S.H. HORI, 1970. – A polygenic study of animal glucose-6-phosphate dehydrogenases. *Jap. J. Genet*, 45 : 319.

MANDULA, B., S.K. SRIVASTAVA & E. BEUTLER, 1970. – Hexose-6-phosphate dehydrogenase : Distribution in rat tissues and effect of diet, age, and steroid. *Arch. Biochem. Biophys.* 141 : 155.

MARKERT, C.L. & F. MOELLER, 1959. – Multiple forms of enzymes : tissue, onto-genetic and species specific patterns. *Proc. Nat. Acad. Sci.* 45 : 753-763.

MARTIN, K.T. & C.W. CAILLOVET, 1976. – Phosphoglucose mutase polymorphism in white shrimp, *Penaeus setiferus*. *Comp. Biochem. Physiol.* 53B : 127-131.

MILLER, P.J. & M.Y. EL-TAWIL, 1974. – A multidisciplinary approach to a new species of *Gobius* (Teleostei : Gobiidae) from Southern Cornwall. *J. Zool.* 171 : 539-574.

NUMACHI, K., 1972. – Genetic polymorphism of tetrazolium oxidase in Black Rockfish Bull. *Jap. Soc. Sci. Fish.* 38 (7) : 789.

OHNO, S., A.W., PAYNE, M. MORRISON & E. BEUTLER, 1966. – Hexose-6-phosphate dehydrogenase found in human liver. *Science* 153 : 1015.

POWERS, D.A., H.M., LENHOFL, & C.A., LEONE, 1968. – Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in coelenterates. *Comp. Biochem. Physiol.* 27 : 139.

ROMER, A.S., 1966. – Vertebrate paleontology, 3rd. ed. University of Chicago Press, Chicago, U.S.A.

SCOPES, K.K., 1968. – Methods for starch-gel electrophoresis of sarcoplasmic proteins. *Biochem. J.* 107 : 139-150.

SHAW, C.R. & E. BARTO, 1965. – Autosomally determined polymorphism of glucose-6-phosphate dehydrogenase in *Peromyscus*. *Science* 148 : 1099.

SHAW, C.R. & KOEN, A.L., 1968. – Glucose-6-phosphate dehydrogenase and hexose-6-phosphate dehydrogenase of mammalian tissues. *Ann. N.Y. Acad. Sci.* 15 : 149.

SHAW, C.R. & R. PRASAD, 1970. – Starch gel electrophoresis of enzymes, a compilation of recipies. *Biochem. Genet.* 4 : 297-330.

SPENCER, N., D.A. HOPKINSON & H. HARRIS, 1964. – Phosphoglucomutase polymorphism in man. *Nature* (Lond.) 204 : 742-745.

SRIVASTAVA, S.K., K.G. BLUME, E. BEUTLER & A. YOSHIDA, 1972. – Immunological differences between glucose-6-p-dehydrogenase and hexose-6-p-dehydrogenase from human liver, *Nature New Biol.* 238 : 240.

STEGEMAN, J.J. & E. GOLDBERG, 1971. – Distribution and characterization of hexose-6-phosphate dehydrogenase in trout. *Biochem. Genet.* 5 : 579.

STEGEMAN, J.J., 1972. – Inheritance of hexose-6-phosphate dehydrogenase polymorphism in brook trout. *Biochem. Genet.* 7 : 279.

STEIN, D.W., ROGERS, J.S. & CASHNER, R.C., 1985. – Biochemical Systematics of the *Notropis roseipinnis* complex (Cyprinidae : Subgenus *Lythurus*). *Copeia* 1 : 154-163.

UTTER, F.M., 1971. – Tetrazolium oxidase phenotypes of rainbow trout (*Salmo gairdneri*) and Pacific Salmon (*Onchorhynchus* spp.). *Comp. Biochem. Physiol.* 39B : 891-895.

WATTS, R.L. & D.C. WATTS, 1968. – Gene duplication and the evolution of enzymes. *Nature* (Lond.), 217 : 1125.

Accepté pour publication le 20.12.85